Biofertilizer with phosphorus; a sustainable and eco-friendly approach for enhancing mungbean growth, productivity and protein content in changing climatic scenario

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# Abstract

The current agro-climatic scenario is full of environmental hazards due to warm and harmful effects of changing climate. Therefore, the application of biofertilizers with phosphorous application is playing a key role in such a harsh conditions for enhancing legumes productivity. Low productivity of grain legumes is generally associated with reduced soil fertility and limited N2-fixation. In order to assess the influence of rhizobium inoculation and phosphorus application on growth and yield of mung bean, an experiment was conducted at Agronomy Research Farm, the University of Agriculture, and Peshawar-Pakistanduring summer 2018. The experiment was laid out in randomized complete block design having three replications with Two factors i.e. seed inoculation (inoculated and un-inoculated) and phosphorus levels (20, 40, 60 and 80 kg ha<sup>-1</sup>). The experimental results showed that seed inoculation with rhizobium performed better than un-inoculated seed in terms of different growth and yield parameters. Rhizobium inoculation significantly enhanced leaves plant-1, plant height, nodules plant, grain yield, and harvest index and protein contents up to (21 %). Regarding phosphorus levels, maximum leaves plant<sup>-1</sup>, nodules plant<sup>-1</sup>, plant height, grain yield, harvest index and protein contents were found superior with the application of phosphorus at the rate of 80 kg ha<sup>-1</sup>. From the experimental results it is concluded that seed inoculation with rhizobium and phosphorus at the rate of 80 kg ha<sup>-1</sup> showed prominent increase in growth and yield and therefore recommended for higher production of mung bean in the agro-climatic condition of Peshawar-Pakistan.

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## Introduction

Mung bean (*Vigna radiata* L.) belongs to fabaceae family and is largely grown in South America, Australia, Africa and South East Asia. It is also amajor kharif pulse of Pakistan. Among grain legumes, mung bean stands second to chickpea (Ali *et al.*, 2004). It is mainly grown as food for human, feed for livestock, green manure and medicinal crop (Ugese *et al.*, 2005). In Pakistan pulses were grown on 0.07% of total cropped area of 22.8 million hectares whereas the total area under mung bean cultivation was 162.5 million hectares and the production was 6.9 million hectares and the production was 6.9 million hectares and the production was 4.5 million tons (MNFSR, 2017).

Rhizobial inoculants are commonly known as an alternative source of nitrogenous fertilizers for legumes (Ali et al., 2010). Biological nitrogen fixation is the second most important biological process after photosynthesis (Hayat et al., 2008) and this fixed nitrogen helps to meet the nitrogen demand of legumes and also for the succeeding crop (Peoples et al., 2009). Soil microorganisms are beneficial because they affect physical, chemical and biological properties of soil. Amongst the soil bacteria there is a unique group called Rhizobia that has a good effect on the growth of legumes. Mung bean also improves the fertility of soil by fixing atmospheric nitrogen into available form with the help of rhizobium species present in nodules of roots. Rhizobia are special soil microorganisms that can form a symbiotic relationship with legumes resulting in biological nitrogen fixation. Rhizobia are soil bacteria which are capable of forming nodules on roots or stems of leguminous plants (Dudeja and Duhan, 2005).

Phosphorus is an essential macronutrient and required for plant growth and various functions like photosynthesis, respiration, storage and transfer of energy, development of root, flowering promotion and fruit ripening (Raboy, 2003; Sangakara *et al.*, 2001). It has been reported by many studies that phosphorus application has great effect on yield of crop and its deficiency limits the response of plant to other nutrients (Akinrinde and Adigun, 2005). It is necessary for regulation of protein synthesis and plays an important role in cell division, tissue formation, root development and hasten maturity. Phosphorus is also an important component of DNA in which genetic data of all living things is present. It improves the quality of crop and also strengthens the root growth and makes it disease resistant. Phosphorus is needed in soil for nitrogen fixation in legumes. It also stimulates the early root growth and also helps in hastening plant maturity and improve quality of seed (Abdullahi *et al.*, 2011).

Keeping in view the importance of grain legumes, the current study was conducted to investigate the optimal level of phosphorus in combination with rhizobial inoculation for higher productivity of mung bean.

## Materials and methods

An experiment was conducted during summer 2018 to analyze the influence of rhizobium inoculation with phosphorus application on growth and yield of mung bean at Agronomy research Farm, The University of Agriculture, Peshawar. The experiment was laid in randomized complete block design (RCBD) with three replications. Plot size was 3 m 2 m contained 6 rows 30 cm apart from each other. Pant to plant distance of 10 cm was maintained in all plots. Mung bean variety Ramazan-92 was sown at the rate of 25 kg ha-1. Four levels of phosphorus (20, 40, 60 and 80 kg ha-1) and rhizobium (inoculated and non-inoculated) were applied. Single superphosphate was used as source of phosphorus. Phosphorus was applied at the time of sowing. Seeds were inoculated with recommended dose of rhizobium before sowing. For seed inoculation first sugar solution was made by mixing 50 g of sugar in 250 ml of distilled water and then applied on seeds of mung bean and then rhizobium was mix with seeds. The mixing of rhizobia with seeds of mung bean was done in cool shaded place and then applied to field at sunset time with a proper moisture content in the field in order to protect the living rhizobia from environmental hazards like warm temperature.

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## Statistical analyses

Data were analyzed statistically using analysis of variance techniques appropriate for randomized complete block design using software staistix 8.1. Means were also compared using LSD test at 0.05 level of probability, when the F-values was significant (Steel and Torrie, 1984).

# **Results and discussion**

## Leaves plant<sup>1</sup>

Rhizobium inoculation and phosphorus levels had significant effect on number of leaves plant<sup>-1</sup> of mung bean. Number of leaves increased with increase in phosphorus application and more leaves were counted in plants supplied with 80 kg P ha<sup>-1</sup> (Fig. 1). Application of phosphorus improves nutrients uptake which consequently improves the plant vegetative growth. These results are in line with the findings of Khan *et al.* (2017) who reported significant increase in number of leaves plant<sup>-1</sup> of mungbean. Likewise, increasing trend in number of leaves plant<sup>-1</sup> was also shown by rhizobium inoculation. The results are similar with that of Bhuiyan *et al.* (2008) who revealed that inoculated plots produced higher number of leaves plant<sup>-1</sup> as compared to uninoculated control.

**Table 1.** Shows leaves plant<sup>-1</sup>, nodules plant<sup>-1</sup>, and plant height (cm) of munbean as influenced by rhizobium inoculation and phosphorus application.

leaves plant-1				Nodules plant-1			Plant height (cm)			
Phosphorus	Inoculated	Un-inoculated	Mean	Inoculated	Un-inoculated	Mean	Inoculated	Un-inoculated	Mean	
20	23	20	22 b	17	12	14 c	69	55	62 bc	
40	25	22	23 ab	23	13	17 b	74	59	66 b	
60	26	22	24 a	24	14	19 ab	75	66	70 a	
80	29	22	25 a	26	15	21 a	83	60	71 a	
	26 a	21 b		23 a	13 b		75 a	60 b		
LSD < (0.05) for P= 1.72			LSD < (0.05)  for  P = 1.59			LSD <(0.05) for P= 5.36				
LSD <(0.05) for RI=1.23			LSD < (0.05) for RI =1.59			LSD <(0.05) for RI= 3.97				
LSD <(0.05) for P x RI= NS			LSD < (0.05) for P x RI= NS			LSD<(0.05) for $P \ge RI = NS$				

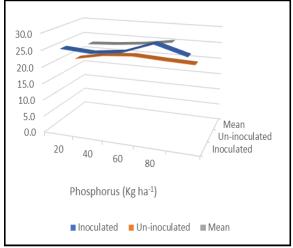
**Table 2.** Shows grain yield (kg ha<sup>-1</sup>), harvest index (%), and protein content (%) of mungbean as influenced by rhizobium inoculation and phosphorus application.

Gr	Grain yield (kg ha-1)				Harvest index (%)			Protein content (%)		
Phosphorus	Inoculated	Un-inoculated	Mean	Inoculated	Un-inoculated	Mean	Inoculated	Un-inoculated	Mean	
20	449	390 c	419	23	20	21 C	19	18	18 d	
40	479	449 b	464	23	22	22 bc	20	18	19 c	
60	611	500 ab	556	27	22	24 ab	22	18	20 b	
80	693	544 a	618	27	25	26 a	23	19	21 a	
	558 a	470 b		25 a	22 b		21 a	18 b		
LSD < (0.05)	LSD < (0.05) for P= 48.05				LSD < (0.05) for P =2.50			LSD <(0.05) for P= 0.85		
LSD <(0.05)	LSD <(0.05) for RI=33.98				LSD < (0.05) for RI =1.76			LSD <(0.05) for RI= 0.60		
LSD <(0.05) fe	LSD <(0.05) for P x RI= NS				LSD < (0.05) for P x RI= NS			LSD<(0.05) for P x RI= NS		

## Nodules plant<sup>1</sup>

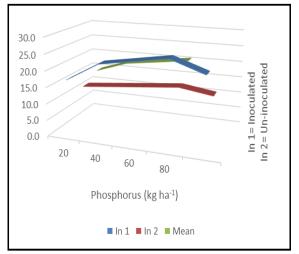
The impact of phosphorus levels and rhizobium inoculation was also found significant for number of nodules plant<sup>-1</sup> of mungbean crop. There was linear

increase in nodules plant<sup>-1</sup> with increase in phosphorus application. Maximum number of nodules plant<sup>-1</sup> were counted in plots treated with higher dose of phosphorus (80 kg P ha<sup>-1</sup>) (Fig. 2).



**Fig. 1.** Leaves plant<sup>-1</sup> of mungbean as influenced by rhizobium inoculation and phosphorus application.

Increase in number of nodules with increase in phosphorus level might be due to phosphorus supply to the roots of plant at various growth stages especially at the time of nodule formation (Ahmad *et al.*, 2015). Similar findings were also observed by Khan *et al.* (2017) who reported that increase in phosphorus levels enhanced number of nodules plant<sup>-1</sup>. Similarly, rhizobium inoculation increased nodules plant<sup>-1</sup> of mung bean rather than without inoculation.

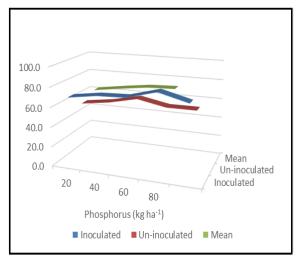


**Fig. 2.** Nodules plant<sup>-1</sup> of mungbean as influenced by rhizobium inoculation and phosphorus application.

The possible reason for increase in nodulation due to inoculation might be that associative effect of bacteria and its activities results in improvement in nodulation. These results are in agreement with the investigations of Tran *et al.* (2007) and Rahman *et al.* (2008) who reported prominent increase in nodules number with rhizobium inoculation.

#### Plant height

Phosphorus application had significant effect on mungbean plant height. Plant height increased with each increment in phosphorus level. Taller plants were noted with 80 kg P ha<sup>-1</sup> (Fig. 3). The increase in plant height with phosphorus might be due to the fact that phosphorus plays essential role in development of root and is vital for respiration, energy synthesis, photosynthesis of plants which results in growth improvement. Jabbar *et al.* (2012) supported our results because they found that increase in levels of phosphorus enhanced plant height of mungbean crop.



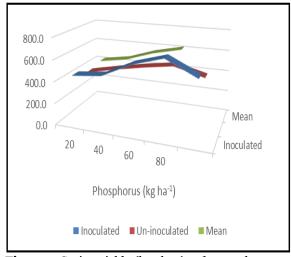
**Fig. 3.** Plant height (cm) of mungbean as influenced by rhizobium inoculation and phosphorus application.

A significant increase in plant height of mungbean was also observed with seed inoculation of rhizobium. Rhizobium enhance nitrogen fixation which is needed vigorous growth and might have caused higher plant height of mung bean. Similar results were also reported by (Ali *et al.*, 2010).

## Grain yield

Rhizobium inoculation and phosphorus application had significantly affected grain yield. Increasing trend in grain yield of mungbean was observed with increasing phosphorus levels (Fig. 4).

Highest grain yield was observed in plots where rhizobium inoculation was applied.



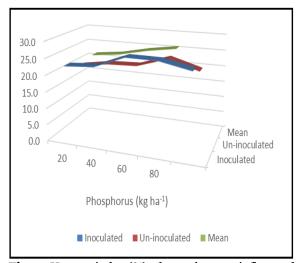
**Fig. 4.** Grain yield (kg ha<sup>-1</sup>) of mungbean as influenced by rhizobium inoculation and phosphorus application.

This increment in grain yield might be due to phosphorus enhance assimilates of plant which are needed for growth and development of the plant (Tariq *et al.*, 2007).

These results are also related with Kumar *et al.*, (2012) who noted that increasing phosphorus levels resulted in maximum grain yield. The results are also in accordance with Khan *et al.* (2015) who showed increase in grain yield with increase in phosphorus level in case of mash bean. Comparing rhizobium inoculation inoculated plots gives more grain yield as compared with un-inoculated plots. The reason for increase might be due to increase number of nodules plant<sup>-1</sup> and dry weight of nodule which result in more accumulation of dry matter during the period of growth and translocate more photosanthate to seed. The results are supported with Bhuiyan *et al.* (2008) who stated increase in yield with inoculation as compared to un-inoculated.

# Harvest index

Harvest index was significantly affected by rhizobium inoculation and phosphorus levels. Maximum harvest index was noted in plots treated with 80 kg P ha<sup>-1</sup> (Fig. 5). Improvement in harvest index might be due to increase in physiological capacity to mobilize photosanthates and their translocation into organs having economic yield. (Saleem *et al.*, 2015). These findings are supported with those of Ahmed *et al.* (2018) who concluded that harvest index increases with increase in phosphorus levels. In case of rhizobium inoculation, inoculated plots showed maximum harvest index than un-inoculated plots. Our results are also supported with the findings of Bhuiyan *et al.*, (2008) who reported maximum harvest index of mungbean with rhizobium inoculation.

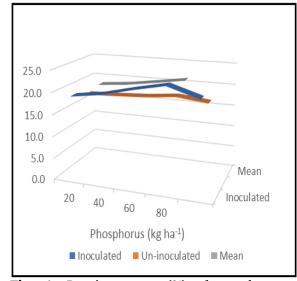


**Fig. 5.** Harvest index (%) of mungbean as influenced by rhizobium inoculation and phosphorus application.

### Protein content (%)

Analysis of variance indicated that protein content (%) of mungbean as influenced by rhizobium inoculation and phosphorus application showed significantly positive response towards treatment applications.

Significantly higher protein content (21 %) was recorded in rhizobium inoculated plots (Fig.6). Rhizobium inoculation enhances protein content in mungbean grains, it might be due to the fact that rhizobium inoculation is a part of nitrogenase reductase enzymes which results improvement of nodules and ultimately enhanced protein content. Tariq *et al.*, (2007) also observed the similar findings. Comparing phosphorus applications, maximum protein content (21.1 %) was observed in plots where phosphorus was applied at the rate of 80 kg ha<sup>-1</sup> while lower protein content (18.4 %) was found in control plots.



**Fig. 6.** Protein content (%) of mungbean as influenced by rhizobium inoculation and phosphorus application.

Increasing phosphorus content in mungbean grains might be due to the phosphorus functional and part of protein synthesis. Kumar *et al.*, (2012) who noted that increasing phosphorus levels results in achieving maximum protein content in mungbean grains.

# Conclusion

On the basis of experimental results, it is concluded that seed inoculation with rhizobium and phosphorus application at the rate of 80 kg ha<sup>-1</sup> resulted maximum growth, grain yield and protein content of mungbean. As per conclusion, it is stated that growing of mungbean for higher productivity and maximum protein content with rhizobium seed inoculation and phosphorus application at the rate of 80 kg ha<sup>-1</sup> of munbean in the agro-climatic condition of the study area are recommended.

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